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Research Report

Iron is a potential key mediator of glutamate excitotoxicity in spinal cord motor neurons

Jixu Yu[a], *Yansu Guo*[a,b], *Mengmeng Sun*[a], *Bin Li*[a], *Yuesheng Zhang*[a,c], *Chunyan Li*[a,b,*]

[a]*Department of Neurology, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei 050000, PR China*
[b]*Institute of Cardiocerebrovascular Disease, Shijiazhuang, Hebei, PR China*
[c]*Department of Cancer Prevention and Control, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, New York, USA*

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ABSTRACT

Threohydroxyaspartate (THA)-induced glutamate excitotoxicity in organotypic culture of rat spinal cord is a well-known model of motor neuron degeneration. THA causes accumulation of synaptic glutamate and over stimulation of the postsynaptic receptor by inhibiting glutamate uptake. This model has also been used to identify agents that inhibit glutamate excitotoxicity by increasing the expression of glutamate transporter. We now show that THA also increases iron level in rat spinal cord tissue, with concomitant modulation of key iron transport and storage proteins, including transferrin receptor, divalent metal-ion transporter 1 and ferritin. More significantly, iron chelator deferoxamine (DFO) was able to completely prevent THA-induced motor neuron degeneration. The protective effect of DFO did not involve enhancing glutamate uptake. These data provide new mechanistic insight into THA-induced glutamate excitotoxicity and suggest that blocking THA-induced iron rise alone may be sufficient for prevention of glutamate excitotoxicity.

1. Introduction

Threohydroxyaspartate (THA) is an inhibitor of glutamate transport and causes glutamate excitotoxicity in organotypic culture of rat spinal cord as a result of blockage of glutamate uptake and ensuring accumulation of extracellular glutamate (Rothstein et al., 1993). THA-induced motor neuron death in organotypic culture of rat spinal cord has been a widely used model of amyotrophic lateral sclerosis (ALS) and was also used for identification of agents that provide neuroprotection by increasing glutamate transporter expression (Rothstein et al., 2005). Glutamate excitotoxicity is thought to result mainly from excess calcium entry into neurons triggered by over-stimulation of postsynaptic glutamate receptors (Choi, 1988). A large rise in intracellular Ca^{2+} levels in these cells causes rapid mitochondrial calcium overload, leading to mitochondrial damage and generation of reactive oxygen species (Ankarcrona et al., 1995; Carriedo et al., 1998; Carriedo et al., 2000), although the precise role of oxidative damage in neuronal death remains undefined.

Iron is known to play an important role in Alzheimer disease, including accelerating amyloid-β aggregation and

* *Corresponding author*. Department of Neurology, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei 050000, PR China. Fax: +86 311 87064024.
E-mail address: hblicy5@yahoo.com.cn (C. Li).

Abbreviations: ALS, amyotrophic lateral sclerosis; DFO, deferoxamine; DMT1, divalent metal-ion transporter 1; IRE, iron-responsive element; IRP1, iron regulatory protein 1; LDH, lactate dehydrogenase; TBARS, thiobarbituric acid-reactive substances; TfR, transferrin receptor; THA, threohydroxyaspartate

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promoting oxidative damage (Casadesus et al., 2004; Castellani et al., 2007; Smith et al., 1997). We suspected that iron might play an important role in glutamate excitotoxicity and neuronal death. Iron is known to potentiate the toxic effects of ROS by catalyzing the formation of highly reactive hydroxyl radicals from hydrogen peroxide through the so-called Fenton chemistry. Thus, while iron is essential for normal physiology (Boldt, 1999), it is also implicated in many pathological processes, including neuron degenerative disorders (Gerlach et al., 1994; Youdim et al., 1993). On the other hand, ROS can convert aconitase to active iron regulatory protein 1 (IRP1) by causing the Fe–S cluster disassembly in aconitase (Pantopoulos and Hentze, 1995). IRP1 binds to the iron-responsive element (IRE) within the stem-loops of transcripts of key iron transport and storage genes (Pantopoulos and Hentze, 1995). If the IRE is located in the 3′ untranslated region of the mRNA, binding of IRP1 usually causes an up-regulation of the coded protein, as with transferrin receptor (TfR) and divalent metal-ion transporter 1 with IRE (DMT1+IRE). Conversely, binding of IRP1 to an IRE in the 5′ untranslated region of the mRNA generally causes a down-regulation, as in the case of ferritin (Danzeisen et al., 2006; Pantopoulos and Hentze, 1995; Rouault, 2006). TfR is expressed on the cell surface and mediates the transfer of extracellular diferric-transferrin to the endosome, where iron is released from transferrin and transported by DMT1 to cytoplasm, and ferritin is a major iron storage protein. Thus, ROS may elevate cellular level of free iron.

In the present study, we have measured tissue iron levels in organotypic culture of rat spinal cord with and without THA-induced glutamate excitotoxicity. Tissue levels of TfR, DMT1 and ferritin were also measured in order to gain further understanding about the potential iron modulation in glutamate excitotoxicity. Moreover, the role of iron in glutamate excitotoxicity was assessed by measuring the protective activity of iron chelator deferoxamine (DFO).

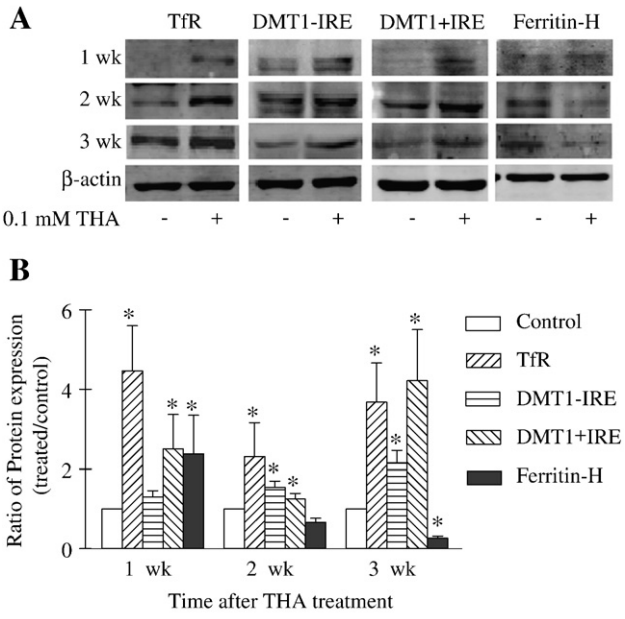
2. Results

2.1. *The expression of key iron transport and storage proteins altered and tissue iron level was increased in THA-induced glutamate excitotoxicity*

Rat lumbar spinal cord explants, after 1 week of culture, were treated with THA at 100 μM for 3 weeks. At the end of the 3-week THA treatment, the explants were harvested for measurement of expression of several iron transport and storage proteins. We found that the expression levels of TfR, DMT1–IRE and DMT1+IRE were all increased significantly after THA treatment, whereas the level of ferritin (only the heavy subunit was measured) decreased significantly (Fig. 1A and 1B). A time course experiment showed that changes occurred rapidly, as increased levels of TfR, DMT1–IRE and DMT1+IRE were detected after 1 week of THA treatment. Interestingly, ferritin-H level increased after 1 week of THA treatment and then declined. The effects of THA on the iron transport and storage proteins, as described above, suggested that THA might elevate tissue iron content. TfR, DMT1–IRE and DMT1+IRE are key cellular iron uptake proteins. Indeed, total iron content in the explants, measured at the end of 3-week of THA treatment increased 21.4% (Fig. 1C, $P<0.05$). Although we were unable to measure free iron due to technical limitations, it seemed likely that tissue free iron level might increase more significantly than the total iron after THA treatment, because ferritin is the main cellular iron storage protein and was markedly down regulated by THA.

2.2. *Iron chelator DFO protects motor neurons against THA-induced toxicity*

DFO is an iron chelator and is widely used for treatment of acute iron poisoning and hemochromatosis. Rat lumbar spinal cord explants, after one week in culture, were pretreated with DFO at 0, 25, 50 and 100 μM for 48 h and then treated with the



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	Total iron (μg/g dry tissue)
Control	96.4 ± 9.8
THA	117.0 ± 7.0*

Mean ± SD (n=4); *different from the control (P<0.05)

Fig. 1 – Effect of THA on iron accumulation and expression of iron transport and storage proteins in explants of rat spinal cord. The explants were treated with either vehicle or THA at 100 μM for 1, 2, and 3 weeks. The media were changed twice each week. (A) Tissue expression levels of TfR, DMT1–IRE, DMT1+IRE and Ferritin-H were measured by immunoblotting. (B) The immunoblotting data are representative of at least three experiments (10–15 explants were pooled in each experiment). β-actin was used as a loading control. Data were expressed as levels of immunoreactivity relative to control values (means ± SD, $n=3–4$). (C) Total tissue iron content was measured by graphite furnace atomic absorption spectrophotometry. *Statistically different from control group ($p<0.05$).